Stadtman Earl 2001 A

Dr. Earl Stadtman Oral History 2001 A

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Earl R. Stadtman, Ph.D.
January 23, 2001

This is the first interview in a series on the career of Dr. Earl R. Stadtman. It was conducted in his office on the second floor of Building 3, National Institutes of Health, Bethesda, Maryland.
The interviewer is Dr. Buhm Soon Park.

Stadtman: I don't know. You understand from the beginning, these things.

Park: Yes.

Stadtman: And have you looked at any of these[1]?

Park: I didn't read very closely. I just took a look at, but I will.

Stadtman: You know, this was a wartime project[2]. And then I started my thesis with Barker, and there are several rather important papers that were published there that had tremendous impact on the mechanism of fatty acid oxidation as well as biosynthesis in the organism that we looked at there. And I don't know... I don't know whether--yeah, I have that checked. That's an important paper because it led to the concept that free fatty acids were not involved in fatty acid synthesis or in oxidation, but, rather, some derivative of the acids was involved. And, subsequently, in studies carried out in Lipmann's[3] laboratory, we showed that coenzyme A was that important component, that it was really the Co-A esters that were implicated in the fatty acid oxidation and fatty acid synthesis.

I think, in retrospect, as far as my own career was concerned, this period was key because it gave me a feeling for what science was all about. And I learned from Barker how to do experiments and to be cautious in the interpretation of your results, that sort of thing. These various papers that I've indicated here are really an outgrowth of that work, which was carried on eventually here, and when we were here in the beginning of the Heart Institute, there were several important reactions that were discovered. One of them was the reaction demonstrated, I mean was the study demonstrating that Co-A esters were capable of undergoing translocation of the Co-A, that Co-A could be passed from one compound to another compound, and that was a very important finding from the standpoint of activation of proteins, I mean of fatty acids for oxidation and other. The other important discovery at that time was that ethyl alcohol was oxidized to acetate, or, rather, acetyl phosphate, but that this was a very complicated process and it involved conversion of the ethyl alcohol to acid aldehyde, and then the oxidation of acid aldehyde was associated with the formation of a Co-A ester, so that instead of getting acetate, as you would expect if you oxidized acid aldehyde, or instead of getting acetyl phosphate, which had been proposed, the primary product, in fact, was acetyl Co-A. So the oxidation of acid aldehyde in the presence of Co-A led to acetyl Co-A. That could then be converted to acetyl phosphate by an exchange reaction in which the phosphate would split the Co-A off and form the acetyl phosphate . And those were important experiments because they called attention to the fact that acetyl phosphates could in fact undergo reactions with Co-A to form the esters of Co-A, which are involved in a number of metabolic processes.

Park: May I ask you one thing?

Stadtman: Sure.

Park: It seems like all the equations are in your mind, and you seem like there is a reaction and then following and following and following, and I want to ask you whether you sort of hypothesized those kinds of mechanisms first and tried to check it with experiments, or you get the insights from the experiments first and then write up the equations.

Stadtman: Both. Both contributed to the development. Some were serendipitous findings. I can give you an example. We were studying the oxidation of butyric acid, which would lead to acetyl Co-A as an ultimate product. And much to our surprise, we didn't find acetyl Co-A. Instead, we found acetyl glutathione, because we had put in glutathione in the reaction mixtures to reduce the Co-A so that it could function in the system, and this led to the discovery of thioltransacetylation process. Acetyl Co-A was in fact the end product of the oxidation, but it had the ability to interact with glutathione and transfer that Co-A to glutathione. And that led to a major project, which was studied in part by Roscoe Brady, who was a member of my laboratory at that time. So that's the thiol ester story. But by the same time, serendipitous things happened. We wanted to see if acetyl acetate derivative was actually formed in the oxidation of butyrate. So I thought, well, if I added hydrogen cyanide in high concentrations, that cyanide would bind to the carbonyl group. You would accumulate, then, the acetoacetyl cyanide complex. When I carried out that experiment, I found, in fact, that didn't happen, but something else happened that I hadn't imagined, and that was that the acetyl group that was formed, the acetyl Co-A that was formed in the oxidation of butyrate, could react with hydrogen cyanide, and it was later shown that that led to acetyl cyanide, which is a very good acetylating agent for amino acids. So we found acetylated amino acids in our reaction mixtures as a consequence of that hydrogen cyanide experiment. And then this led, as I've indicated in some of these checked references, to the further studies on the reaction by Eggerer[4], who was a postdoc in my lab, and we still don't know why acetyl cyanide reacts with acetyl CoA. It's an enzymatic reaction; it's an enzyme-catalyzed reaction between acetyl Co-A and cyanide to form acetyl cyanide. We don't know what the normal function of that enzyme is, but it's a curious reaction. And the same sort of thing happened with imidazole. We were using imidazole as a buffer in some of our experiments, and much to our surprise, we accumulated acetyl imidazole, again because there's an enzyme that can transfer the acetyl group from acetyl Co-A to imidazole. And this is a very high-energy compound. So, what is the normal function of that enzyme? We don't know.

Park: You seem to be quite open to unexpected results in your experiments.

Stadtman: Yeah. And...

Park: And how many times do you do the same experiment to confirm your experimental results?

Stadtman: Oh, I usually repeat most every experiment that is unexpected. I would repeat it several times, three or four times, to make sure that it's in fact real and look at it from several different points of view. But that, I think, you may have realized because I think it was Prusiner[5], when he came and gave a talk here at NIH, he said that while he was a postdoc in my lab, he found that I insisted that he repeat the same experiment over and over and over again in order to prove that it was actually so. Now, I can tell you that my research shifted from fatty acid metabolism, but the reason was so that in my own mind, I knew that this study had started in Barker's laboratory, and Barker was a very outstanding scientist, a wonderful teacher, and just everybody knew Barker, but relatively few people knew me. So I assumed that if I continued to work on this area, that I would always be identified with Barker, and I wanted to prove that I could do something of substance by myself. So I shifted fields a little bit and I took advantage of my teachers in teaching about isolation of organisms from the soil by an enrichment culture technique that would enable you to find organisms that had a unique ability to metabolize the compound that you provided as the major substrate. So what you do is make up a mineral media, and then to that, if you wanted to study, for example, the oxidation of riboflavin, which is one of the things I was interested in at the time, you'd put in riboflavin as the only carbon and nitrogen source. And from this mud, there would be organisms that would grow under those conditions, and they grew by decomposing riboflavin. So you could isolate from the soil the pure culture of an organism that had this unique ability, and that was a technique which Barker had introduced me to when I was a graduate student, and he in turn had learned from Kluyver who was a very important Dutch biochemist, microbiologist[6]. But anyway, the soil-enrichment technique was then used to study a number of systems, and among others, I was interested in heterocyclic compound decomposition, of which riboflavin was used as one component, and another study was carried out on the oxidationof nicotinic acid. And Ira Pastan, whom you may know, was the postdoc in my lab, and I asked him to set up soil-enrichment cultures using nicotinic acid as the sole source of nitrogen and carbon, and he isolated such an organism, which later proved to be very important in understanding the role of vitamin B12 and some other factors. My wife also took over the problem and continued to work on it for some years. And, in fact, the organism which he isolated, which was an enterobacterium, we named Clostridium barkeri in honor of our teacher, Barker. Then, Vagelos came as a postdoctoral fellow in the lab, and he started working on propionic acid metabolism, which is something that I had shifted to, and that was kind of a serendipitous thing also. I wondered what acrylyl Co-A, what it would, whether it would form a hydroxyl derivative, and just by addition of water across the double bond. And much to my chagrin, I found that ammonia added across the double bond to form a beta alanine as a product. But anyway, as a follow-up to that work, Vagelos became interested in the propionic acid metabolism, and this led back to fatty acid synthesis again, and he discovered an important reaction that, although acrylyl Co-A and propionyl Co-A did not seem to fit into the long-chain fatty acid synthesis system, they did undergo CO_2 exchange or reactions when propionyl Co-A would react with CO_2 and you'd end up with methylmalonyl Co-A, which was the real intermediate in long-chain fatty acid synthesis. He spent several years working on that. Then he left here and went to St. Louis, where he was chairman of the Department of Biochemistry there, and he continued that work and worked out the whole mechanism of fatty acid oxidation used in the complex of enzymes. I already told you about... Oh, no. This was something else again. Then I took a sabbatical and I went to Lynen's[7] laboratory, a good friend of mine, and who worked in the field of acetyl Co-A for many years. And there I...

Park: In Paris?

Stadtman: No. This was in Munich.

Park: Oh, Munich.

Stadtman: And while I was there, he asked me what did I want to work on, and I told him that I had just read some interesting papers on the anti-conversion of succinyl Co-A and methylmalonyl Co-A and that there was a suggestion that it might be a reaction involving vitamin B12 coenzyme and that I would like to look at that. And so I spent half a year in his lab studying that and, in fact, was able to isolate the enzyme that catalyzed the conversion of methylmalonyl Co-A to succinyl Co-A, and indeed showed that vitamin B12 coenzyme is required for that reaction. And that has stimulated an interest in vitamin B12 in the laboratory, not only by me, but other workers in the laboratory for some time thereafter. And among other things, the metabolism of nicotinic acid turned out to have a step that required B12. So this was the mechanism of coenzyme-dependent conversion. And then, in a later study, vitamin B12 was shown to be the coenzyme for the oxidation of fatty acids with uneven number carbon atoms. That was the outgrowth of that work. After that first six months, I went to George Cohen's laboratory in Paris, and that's where we are here. And he was working at that time on how cells regulate branch pathways, and it was known that feedback inhibition was involved, but he didn't know the details, and so he asked me to work on aspartokinase, which was one of the enzymes that was involved in the biosynthesis of different pathways. The first step--its metabolism was the first step in a number of metabolic pathways. And what I found was that there wasn't one aspartokinase, but in fact there were three aspartokinases, and that each aspartokinase was regulated by feedback inhibition of the final product of that unique pathway. And so this led to the idea that branch pathways were regulated by feedback control as well as by feedback inhibition of synthesis, which is also a factor.

Then I came back to the United States and I thought I was going to continue working on the nicotinic acid story, which I had left when I went to Europe. And during that period I got an application from a young man who had worked with Helen Whiteley, who is a very good microbiologist out in Seattle, and he said he'd like to come as a postdoctoral fellow, and I accepted him[8]. And when he arrived, he said, "What should I work on?" and I said, "Well, you know, we're working on this nicotinic acid story," and he said, "Oh. I thought maybe it would be possible to work on the aspartokinase," as a continuation of what I had been doing in George Cohen's laboratory. So I told him, "Well, you know, that's really George Cohen's problem. I don't want to become a competitor with him in that field. If you want to work on a branched metabolic pathway, then you'll have to find another enzyme." So I suggested that he go to the library and look at metabolic pathway charts and whatnot and come up with suggestions of enzymes that were involved in not one but several metabolic pathways. And I also told him that it had to be an enzyme whose activity could be readily measured. Well, he came back with three ideas. One of them was glutamic dehydrogenase, which is involved not only in protein synthesis, but it forms -ketoglutarate, which has a role in many metabolic functions in the cell. I thought that was a good suggestion, so I told him... And the other thing he came up with was glutamine synthetase. And the third one that he suggested was phosphoribosyl pyrophosphate synthetase. All of these had been shown to be involved in more than one metabolic pathway. So I asked him to start on the glutamic dehydrogenase because that's the simplest of all to measure, and he did, and he very quickly proved that it had no feedback characteristics, so it was not interesting from the standpoint of feedback control, allosteric control, if you will. So then he turned to glutamine synthetase, and after a very short time, he was able to show that it was an enzyme that was regulated by five different end products of glutamine and glutamine metabolism. So that got us started on a new activity in trying to unravel the regulation of this enzyme. Unlike aspartokinase, there was only one enzyme, but it turned out that that enzyme was regulated by what we subsequently referred to as cumulative feedback inhibition. Each one of the end products could inhibit that enzyme partially but not completely, but when you had all of them there, then they would virtually completely inhibit the activity of the enzyme, so there was a complementary thing. And then we showed that the primary step following this was subject to control by other factors, but the important point was that this enzyme had the unique ability to be regulated by numerous end products of its metabolism. And so we started working on that, and the--after he... He actually crystallized the enzyme, crystallized it and studied the properties of the enzyme, established that it was a dodecamer composed of 12 identical subunits. After he had carried out a large amount of study on this, he left and went to Irvine[9], and another person came to the laboratory[10], and I asked him to continue this work. Well, we had almost run out of the purified preparation that Woolfolk had made, so he grew up another batch of cells, *E. coli* we were using, to study further and go through the same purification procedures, which he did. But much to our chagrin, his preparation didn't exhibit all of that inhibition. So here we were in the middle of a controversy, if you will, and I was sure that there was nothing wrong with the other study because I had participated directly in part of it and confirmed the thing. But then I suggested that he compare the UV spectrum of the two purified preparations. We still had a little of the other left. And much to our surprise, we found that the new preparation that he had made had an absorption band at around 280 and at 260 nanometers, suggesting that some nucleotide was associated with the new preparation that was not associated with the other. This led to the demonstration that the enzyme was subject to control by a mechanism which involved transfer of the adenylic acid moiety of ATP to a tyrosine residue on the protein, and that altered the allosteric properties of the enzyme's feedback inhibition. And, of course, it was clear that if there was adenylylation[11] glutamine synthetase and it had a regulatory function, then there had to be an enzyme that would remove that adenylic acid residue. So we looked for that and found, in fact, that there was such an enzyme.

It was purified and studied, and this led to a further discovery, and that is that the ability to adenylylate and deadenylylate the enzyme was also under control by another protein, and that protein was a protein whose activity was also controlled by interconversion between two forms, but in that case, instead of adenylylation, it was urdidylylation, starting with UTP. And, again, the uridyl group was attached to a tyrosine residue on the regulatory protein. And then this led to a very complicated story showing that not only the adenylyl transferase was itself regulated by that protein, but its capacity to be regulated by that protein was dependent upon other metabolites, and the same was true for the deadenylylation. There were metabolites required for that, and these were identified, -ketoglutarate being one, and ATP was a regulator. There were several metabolites that regulated that activity. So that got us into the field of cellular regulation, and together with [P. Boon] Chock, we did a lot of work on the kinetics and also on theoretical analysis of interconvertible enzyme cascades.

Park: When did you use the term cascade?

Stadtman: The what?

Park: When did you start using the term cascade?

Stadtman: We called it a cascade because it was one reaction on top of another.

The term cascade had been introduced earlier by what's his name, I can't think of his name now. But he was studying blood clotting, and he referred to a blood-clotting cascade because there's a series of events that are involved in that. And so we called this a cascade, a cyclic cascade, or a cascade of cycles, if you will, because you had the uridylylation and uridylylation cycle superimposed on the adenylylation cycle. And the curious thing about that analysis, it showed that these cascades had unique characteristics that one would not have expected. Among other things, they could exhibit a kind of a logarithmic increase. It's really a lag phase going up exponentially as far as depending upon the effector concentrations. Sigmoidal type of kinetics could be operated. It showed that you could get enormous amplification in the signaling process . A little bit of signal could cause a big change in the overall process, so there was a rather unique property of these cascades. I think that today people don't fully realize, but, you know, signal transduction has become the thing today. But I think they don't really appreciate that those are really cyclic cascades that are involved, and therefore they have properties that are unique, that are different from what one might imagine. And, in fact, the adenylylation of a tyrosine residue was in fact the first example of a tyrosine modification used for regulatory functions. Unfortunately for us, it was an adenyl group, not a phosphate moiety, or we would have been recognized as the first to show phosphorylation of a tyrosine residue in protein from a regulatory point of view. But anway, that changed...

Park: Did you mark the papers, important papers?

Stadtman: Well, let's see. I hadn't... This is the cyanide story I told you about.

Park: Yes.

Stadtman: And this is the riboflavin thing I mentioned. This, the first on cumulative feedback inhibition, what we called it, showing multiple end products regulated glutamine synthetase. This is the nicotinic acid thing that I went over briefly. That's another story I'll have to come to. This is also important because it gives some details on the nicotinic acid metabolism by Pastan and [Lin] Tsai, who is still here. There are a lot of things here, but I am trying to stick to this story that I just told you about. The question is, where does the... Ben Shapiro was an important person who did a lot of the work on the deadenylylation of glutamine synthetase, and he, after that, he left and became, eventually, chairman of the Department of Chemistry in Seattle, and then he was recruited by Merck, and he's now vice president of Merck in charge of research worldwide. That's his official title. This is the deadenylylation enzyme. These were all relevant to what I told you. You can tell from the titles. But now I want to get down to the... As far as the deadenylylation story is concerned, this is a key paper because it was demonstrating the uridylylation] of this regulatory protein that I was telling you about. And that work was carried out by Brown[12] and [Amiel] Segal.

As you know, Brown got the Nobel Prize. Siegel was a very good investigator, but he chose to go to back to medicine, so he's one of the few postdocs that I've had who came as M.D.s and stayed in medicine. Most of them went into research.

Stadtman: Here we go. Interconvertible enzyme cascades in metabolic regulation. So these were all about this interconvertible enzyme things. Now, then...

Park: So you almost went on more than a decade?

Stadtman: Oh, yes. It was... See, this was published in 1979 and discovery must have been at least 10 years earlier. Well, '64. It was fifteen years.

Well, then, just to give you a little background, there are a lot of specifics in here, different things. For example, I wanted to know more about one-carbon metabolism, so I suggested to one of my postdocs that he set up an enrichment culture in which he put in methylamine as the only carbon and nitrogen source, so if the organism grew, it would have to grow on a one-carbon compound. And he, in fact, isolated an organism that did that, and studied its chemistry in some detail, and then it was picked up by other workers that came to the lab and continued.

Park: You may stop anytime you want for today's session. I can come back any time.

Stadtman: Yeah, okay.

Park: If you feel tired or you...

Stadtman: I'm not tired, but there's only one other point that I might make.

I can go through these in more detail on another occasion if you like, but I think we're running out of time and it's already almost an hour since Terry was going to see you, so I'd better let you go talk to her. But I can do it again if you want any time, and we can pick up some of the other things.

I do want to get to the story of why we are doing what we're doing now.

Park: Yeah, a fascinating story. I really got into the story of a cascade and the whole story of how you discovered the process in stepwise...

Stadtman: That's the way it developed.

Park: Yes. It's really interesting.

Stadtman: Somewhat serendipitous. You make an enzyme preparation and you show that it does one thing. Then you make another one that doesn't behave quite the same, and then you're on to something else.

Park: Right. In a textbook, the cascade is so elegant and it's just there, but it took a lot of time...

Stadtman: Absolutely.

Park: ...and experiments.

Stadtman: Yeah, a lot of experiments.

Park: So I want to talk with you about that more. But for today...

Stadtman: I'm sorry I took so much time.

Park: Oh, no, no, not at all.

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[1] Buhm Soon Park is referring to Earl's earliest publications from his years at Berkeley as an assistant and graduate student with Horace Barker. Earl, Terry, and Bob Switzer wrote the (post-mortem) summary of Barker's work for the National Academy, as Barker was a member. See:H.A. Barker. By Switzer RL, Stadtman ER, Stadtman TC.

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[2] The study of the browning of apricots. The War Department wanted to send dried California apricots to the troops in the Pacific for their nutritional health. Barker's lab undertook studies on the chemistry of the browning, on methods for preserving the apricots, mainly with sulfur, and the maximum amount of sulfur that soldiers would tolerate and still eat the preserved apricots.

[3] Fritz Lipmann. Awarded the Nobel prize in 1953 for the work Earl mentions. The citation read, "for his discovery of co-enzyme A and its importance for intermediary metabolism"

[4] Hermann Eggerer

[5] Stanley Prusiner. Awarded the Nobel prize in 1997 for the discovery of prions (infectious proteins)

[6] Barker used the enrichment culture technique to isolate a bacterium that could, among other metabolic feats, synthesize fatty acids. He isolated the bacterium from Dutch soil while he was in Delft working in the laboratory of Kluyver. Barker eventually named it *Clostridium kluyverii* in honor of Kluyver.

[7] Feodor Lynen. Awarded the Nobel prize in 1964

[8] Clifford A. Woolfolk

[9] He actually went to UC Irvine

[10] Bennett M. Shapiro

[11] This is the correct terminology and correct spelling – it has 2 “yl”’s. Earl knew that this was the appropriate term and always used it. Other investigators who were less familiar with the rules of nomenclature often spelled it “adenylation”.

[12] Michael Brown. Awarded the Nobel prize in 1985.